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Discovery of a New Role of Human Resistin in Hepatocyte Low-Density Lipoprotein Receptor Suppression Mediated in Part by Proprotein Convertase Subtilisin/Kexin Type 9

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Objectives In this study, our goal was to determine if human resistin plays a role in regulating the uptake of atherogenic low-density lipoproteins in human hepatocytes.

Background Serum levels of resistin, an adipose tissue–derived adipokine, are increased in human obesity and are positively correlated with atherosclerotic cardiovascular diseases. However, the function of resistin in humans is enigmatic.

Methods Human hepatocytes (HepG2 and primary) were treated (24 h) with the following: 1) purified human resistin at various concentrations, with and without lovastatin; and 2) obese human serum with elevated resistin levels or serum from which resistin was removed via antibody-immunoprecipitation. The effect of the treatments on cellular low-density lipoprotein receptor (LDLR) and proprotein convertase subtilisin/kexin type 9 (PCSK9) messenger ribonucleic acid and protein levels were determined by using real-time polymerase chain reaction and Western blotting, respectively.

Results Resistin, at physiological levels observed in human obesity, down-regulated hepatocyte LDLR expression substantially (by 40%). A key mechanism by which human resistin inhibited LDLR levels was by increased cellular expression of the recently identified protease, PCSK9, which enhances intracellular LDLR lysosomal degradation. The quantitatively important role of human resistin in LDLR expression was demonstrated by antibody-immunoprecipitation removal of resistin in human serum, which decreased serum stimulation of hepatocyte LDLRs markedly (by 80%). Furthermore, resistin diminished statin-mediated up-regulation of the LDLR by 60%, implicating resistin in the relative ineffectiveness of statins in selective target populations.

Conclusions These results reveal for the first time that resistin is a highly attractive therapeutic target in ameliorating elevated serum low-density lipoprotein and, thereby, atherosclerotic cardiovascular diseases in obese humans. (J Am Coll Cardiol 2012;59:1697–705) © 2012 by the American College of Cardiology Foundation

Obesity is a growing global epidemic, with more than 400 million obese adults worldwide (1). The consequences of obesity are dire, as obesity is a major cause of metabolic syndrome, leading to atherosclerotic cardiovascular diseases (ASCVD), with high associated morbidity and mortality rates (2,3). One of the earliest and most prevalent metabolic impairments in obesity, comprising the metabolic syndrome, is dyslipidemia (3,4). Dyslipidemia in obesity includes elevated serum levels of atherogenic apolipoprotein B–

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Abbreviations and Acronyms

ASCVD = atherosclerotic cardiovascular diseases

BMI = body mass index

HMG-CoA = 3-hydroxy-3-methylglutaryl–coenzyme A

LDL = low-density lipoprotein

LDLR = low-density lipoprotein receptor

mRNA = messenger ribonucleic acid

MTP = microsomal triglyceride transfer protein

PCSK9 = proprotein convertase subtilisin/kexin type 9

siRNA = small interfering ribonucleic acid

SREBP2 = sterol-responsive element-binding protein 2

containing particles, particularly low-density lipoprotein (LDL). Elevated numbers of LDL particles comprise a major component of the characteristic and common dyslipidemia in human obesity, known as the lipid triad or atherogenic dyslipidemia, which also includes high serum triglycerides and low serum levels of high-density lipoprotein cholesterol (5,6).

The lipid triad is most highly prevalent in obese individuals with central or visceral abdominal obesity, the most metabolically adverse type of obesity, which engenders the highest risk of ASCVD (5,6). The lipid triad overall greatly raises the risk for myocardial infarction, coronary heart disease, and ASCVD in general.

The component of the triad involving LDL delineates that although LDL particle numbers are characteristically increased in obesity, reflected by elevated serum apolipoprotein B levels (7), the LDL particles themselves are typically small and dense and thus contain lower lipid levels. Thereby, serum LDL cholesterol levels are not always elevated in obesity, although LDL cholesterol is usually at least moderately elevated, and in many cases, highly elevated in obesity (6,8,9). Nonetheless, elevated numbers of LDL particles, or elevated apolipoprotein B, are strong ASCVD risk factors and play a major role in the initiation and development of atherosclerosis (6,9,10), as they result in increased uptake of atherosclerotic LDL particles into the vascular wall, where they are retained (7). In fact, elevated circulating LDL is both necessary and sufficient to promote ASCVD (11).

However, our understanding of the mechanisms responsible for elevated LDL in obesity is only rudimentary. Overproduction of apolipoprotein B–containing particles, including LDL, by the liver can partially explain the elevation in serum LDL levels in obesity (12). However, in most metabolic states, regulation of circulating LDL levels is controlled in large part by the rate of liver uptake and clearance of LDL particles by hepatocyte cell surface low-density lipoprotein receptors (LDLRs) (12–14). Indeed, decreased expression of LDLRs and decreased catabolism, uptake, and clearance of LDL particles have been shown to be major contributors of elevated serum LDL in obesity, especially visceral obesity (12,15,16). Despite the central role of the LDLR in regulating circulating LDL concentrations, few physiological factors have been identified that directly affect hepatic LDLR levels.

We identify here a major role for resistin, an adipose tissue–derived adipokine, in human hepatocyte LDLR expression, examining a previously undescribed relationship between obese adipose tissue and impaired hepatic LDL regulation. Resistin is a member of a class of small cysteine-rich secreted signaling proteins, collectively termed resistin-like molecules (17). Resistin expression in adipose-tissue adipocytes and macrophages is increased in obesity, leading to increased circulating resistin levels (18,19). There are also strong positive correlations between plasma resistin levels and increases in body mass index (BMI) and visceral fat (18–20). Conversely, in overweight and obese humans, serum resistin levels have been found to consistently decrease with weight loss attributed to controlled exercise or hypocaloric diets (21–23). Finally, the importance of resistin as an ASCVD risk factor in obesity is highlighted by the fact that serum resistin levels in humans are predictive of coronary atherosclerosis, independent of the presence of obesity (24).

Our key finding that resistin, at physiological levels observed in human obesity (50 ng/ml) substantially reduces human hepatocyte expression of LDLRs (by 40%) (Fig. 1A), suggests that elevated resistin is a key pathophysiological link between obesity and elevated LDL and atherogenesis in humans. Such resistin-mediated reductions in hepatocyte LDLRs, which we identified in both HepG2 cells and primary human hepatocytes isolated from fresh human livers, would be expected to greatly deteriorate the dyslipidemic profile of obese individuals (25).

Cross-talk between adipose tissue and metabolically active tissues, such as the liver, as described earlier, has been noted before and has been shown to be mediated by adipocyte-derived adipokines and cytokines (25). Resistin-mediated impairment of hepatocyte LDLR delineates a new physiological/molecular pathway linking adipose tissue adipokines and hepatic metabolism. In this study, our goal was to determine if human resistin plays a role in regulating the uptake of atherogenic LDL in human hepatocytes.

Methods

For a supplementary description of the Methods section, please see the online version of this paper.

Results

Dose-response experiments with human resistin in human hepatocytes. Cultured human hepatic HepG2 cells (American Type Culture Collection) were maintained in 1% fetal bovine medium–Dulbecco's modified Eagle's medium and treated with recombinant purified human resistin (Calbiochem, Nottingham, United Kingdom), confirmed via denaturing sodium dodecyl sulfate polyacrylamide gel electrophoresis (10%) using a rabbit polyclonal antibody against human resistin (Santa Cruz Biotechnology, Santa Cruz, California) for detection. A single 12-kDa band was observed in our resulting immunoblot, confirming that our resistin source was indeed purified human resistin.

Cryopreserved platable primary human hepatocytes, metabolism qualified from multiple normal human donors,

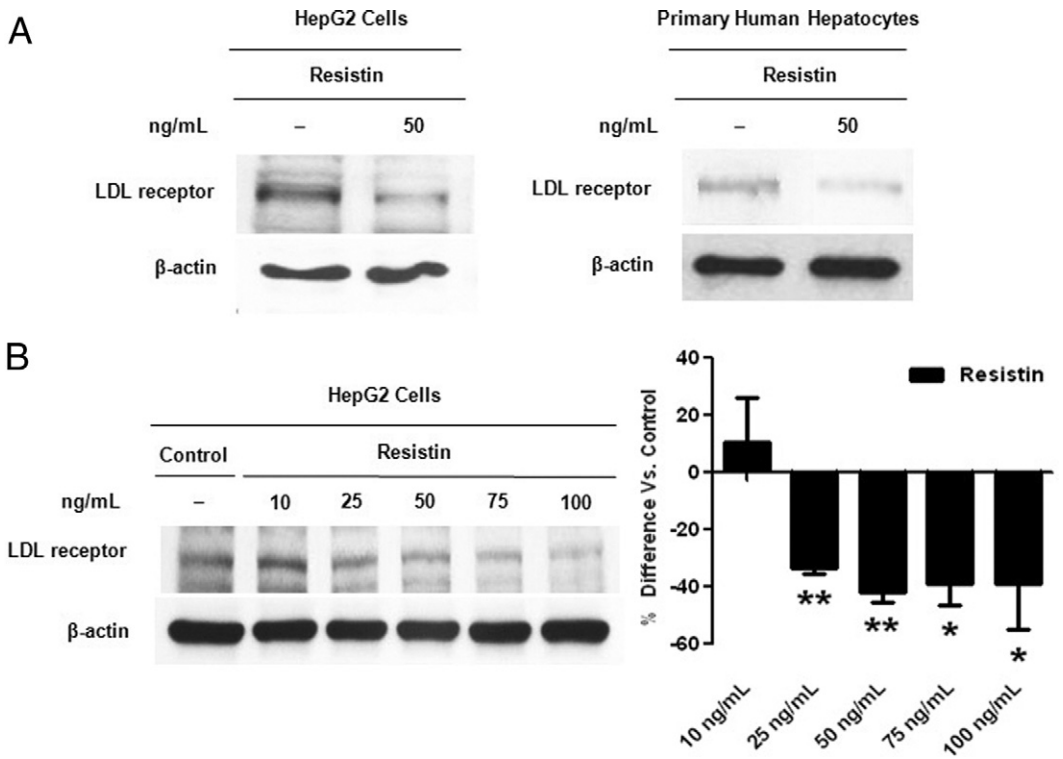

Figure 1 **Examination of the Effect of Human Resistin on Human Hepatocyte LDLR Protein Levels**

(A) Treatment of HepG2 cells and primary human hepatocytes for 24 h with resistin, at the level characteristic of obese human serum (50 ng/ml), markedly inhibited the cellular expression of low-density lipoprotein receptor (LDLR) protein (compared with untreated control cells). **(B)** Resistin reduced cellular LDLR protein levels in HepG2 cells in a dose-responsive manner. $*p < 0.05$; $**p < 0.01$.

were obtained from CellzDirect/Invitrogen, Inc. (Durham, North Carolina). The cells were incubated in the manufacturer's incubation media for 16 h before human resistin 50 ng/ml treatment for 24 h.

All of the experiments to follow were performed at least 3 times, and representative results are shown. Beta-actin or glyceraldehyde-3-phosphate dehydrogenase were used as internal controls to confirm equal protein loading in our Western blots.

Dose-response experiments were performed in HepG2 cells with 0, 10, 25, 50, 75, and 100 ng/ml of human resistin for 24 h. Western blots were then performed on cell lysates to detect the cellular expression of LDLR protein (Figs. 1A and 1B). Significant reductions in HepG2 LDLR protein expression, compared with untreated control cells, were observed at 25- and 50-ng/ml resistin treatments ($p < 0.01$) and at 75- and 100-ng/ml resistin treatments ($p < 0.05$) (Fig. 1B). The significant decline in LDLR protein expression with resistin treatment of HepG2 cells at 50 ng/ml (40 ± 2%) was confirmed in primary human hepatocytes (21 ± 6%) ($p < 0.05$) (Fig. 1A).

Mechanisms by which resistin mediates a reduction in human hepatocyte LDLR levels. Treatment of HepG2 cells with human resistin (50 ng/ml, 24 h) increased cellular sterol-responsive element-binding protein 2 (SREBP2) messenger ribonucleic acid (mRNA) expression by 3-fold and the mRNA expression of SREBP2 target genes, including 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase, the LDLR, and proprotein convertase subtilisin/kexin type 9 (PCSK9) ($p < 0.05$ for all versus untreated control cells) (Fig. 2A). Stimulation of HMG-CoA reductase by resistin would be expected to increase cellular de novo lipogenesis, and, indeed, there was an increase in intracellular neutral lipid content, as assessed via hematoxylin/Oil Red O staining of hepatocytes (Fig. 2B). Gas chromatography analysis of lipids extracted from resistin-treated hepatocytes showed significant increases in cellular triglyceride ($p < 0.01$) and cholesteryl ester ($p < 0.05$) contents by 24 ± 1% and 18 ± 4%, respectively (Fig. 2C).

Hepatocyte PCSK9 protein levels were assessed in response to resistin (50 ng/ml, 24 h) treatment. In HepG2 cells, resistin caused a 40 ± 2% increase in PCSK9 expression, and in primary human hepatocytes, resistin caused a 30 ± 5% increase in PCSK9 protein expression ($p < 0.01$ for both) (Fig. 2D).

Because PCSK9 is known to induce intracellular degradation of the LDLR (13,14), our goal was to determine if the resistin-mediated decline in hepatocyte LDLR protein levels involved PCSK9. PCSK9 small interfering ribonu-

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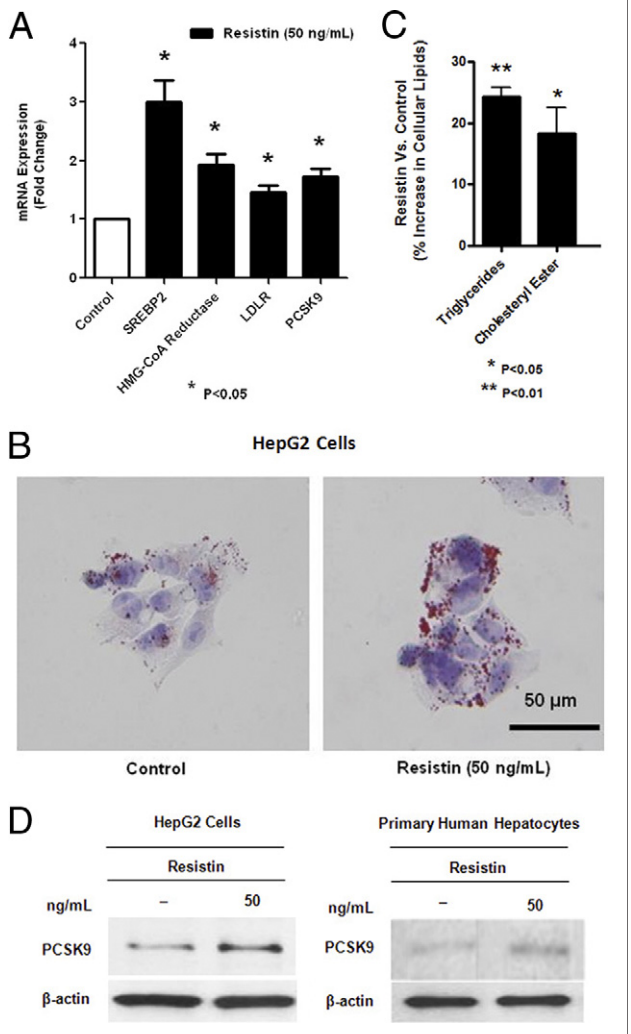

Figure 2 **Mechanisms Accounting for Reduced Hepatocyte LDLR Protein Expression by Resistin Treatment (50 ng/ml, 24 h) of Human Hepatocytes**

(A) Resistin significantly increased messenger ribonucleic acid (mRNA) levels of sterol-responsive element-binding protein 2 (SREBP2) and its target genes in HepG2 cells (versus untreated control cells). **(B)** HepG2 cells treated with resistin showed greater accumulation of neutral lipids versus untreated control cells. **(C)** HepG2 cells after treatment with human resistin showed significant increases in cellular neutral lipids. **(D)** Resistin markedly increased cellular proprotein convertase subtilisin/kexin type 9 (PCSK9) protein levels in HepG2 cells and in primary human hepatocytes. LDLR = low-density lipoprotein receptor; HMG-CoA = 3-hydroxy-3-methylglutaryl–coenzyme A.

cleic acid (siRNA) treatment of HepG2 cells for 24 h inhibited PCSK9 mRNA levels significantly by 60 ± 2% ($p < 0.01$) compared with vehicle control hepatocytes incubated with transfection reagent alone. Treatment with small interfering PCSK9 caused a large elevation (114 ± 15%) in hepatocyte LDLR expression versus untreated control cells, which was ablated with resistin treatment (50 ng/ml) (Fig. 3A). Further determination of cellular PCSK9 protein levels in response to PCSK9 siRNA treatment displayed a 49 ± 1% decline in PCSK9 protein ($p < 0.01$), an effect that was minimized by the addition of resistin (50 ng/ml) to a 31 ± 4% decline in PCSK9 protein levels (Fig. 3B).

Quantification of the role of PCSK9 in the resistin-induced reduction in hepatocyte LDLR levels. Hepatocytes were isolated ex vivo from wild-type and PCSK9 gene-deleted mice, treated with resistin (50 ng/ml, 24 h), and then assessed for LDLR and PCSK9 protein levels by Western blot. In hepatocytes from wild-type mice, resistin markedly decreased LDLR protein levels by 37 ± 6% compared with untreated hepatocytes ($p < 0.01$) and also increased PCSK9 protein levels by 52 ± 8% ($p < 0.01$) (Figs. 3C and 3D); however, in PCSK9 knockout mice, resistin only decreased LDLR levels by 16 ± 1% ($p < 0.01$) (Fig. 3C).

Effect of resistin on hepatocyte LDLR levels in the presence of a microsomal triglyceride transfer protein inhibitor. Microsomal triglyceride transfer protein (MTP) inhibitors, as a class, reduce the egress of cellular lipids, thereby causing cellular accumulation of lipids (26). This action should prevent the stimulation of SREBP by resistin and determine whether the resistin-mediated reduction in LDLR protein levels are dependent on SREBP2 stimulation of LDLR expression. MTP inhibition (via CP-346086 at a nontoxic dose that did not reduce cell viability [1.3 nmol/l]) did induce a significant reduction in SREBP2 mRNA expression and its target gene, HMG-CoA reductase ($p < 0.05$ for both) (Fig. 4A). This reduction in SREBP2 by CP-346086 reversed the increase in PCSK9 (Fig. 4B) and the decline in LDLR protein levels observed with resistin treatment (Fig. 4C).

Effect of resistin on hepatocyte LDLR levels in a physiologically relevant human setting. HepG2 cells were incubated with serum (10% in Dulbecco's modified Eagle's medium for 24 h) from healthy obese males (BMI >30 kg/m^2 and <35 kg/m^2 and waist circumference >102 cm) ($n = 13$) with high resistin concentrations (62% elevated compared with lean subjects) ($p < 0.01$) (Fig. 5A) and compared them with HepG2 cells incubated with serum from lean males (BMI ≤25 kg/m^2 and waist circumference <102 cm) ($n = 10$). The results demonstrated a significant 31 ± 5% inhibitory effect of obese human serum on cellular LDLR protein expression versus lean serum incubation of hepatocytes ($p < 0.01$) (Fig. 5B), and a 39 ± 11% elevation in PCSK9 levels with obese versus lean serum incubation of hepatocytes ($p < 0.01$) (Fig. 5C).

We then performed antibody-immunoprecipitation removal of resistin from human serum using a polyclonal antibody directed against human resistin, and examined the subsequent effect on hepatocyte LDLR and PCSK9 expression. Antibody removal of resistin in obese human serum reversed the obese serum-mediated reduction in cellular LDLRs by 76 ± 14% ($p < 0.01$) (Fig. 5D). Removal of resistin in lean human serum also increased hepatocyte LDLR levels by 54 ± 11% ($p < 0.01$) versus lean serum incubation of hepatocytes. Antibody removal of resistin in

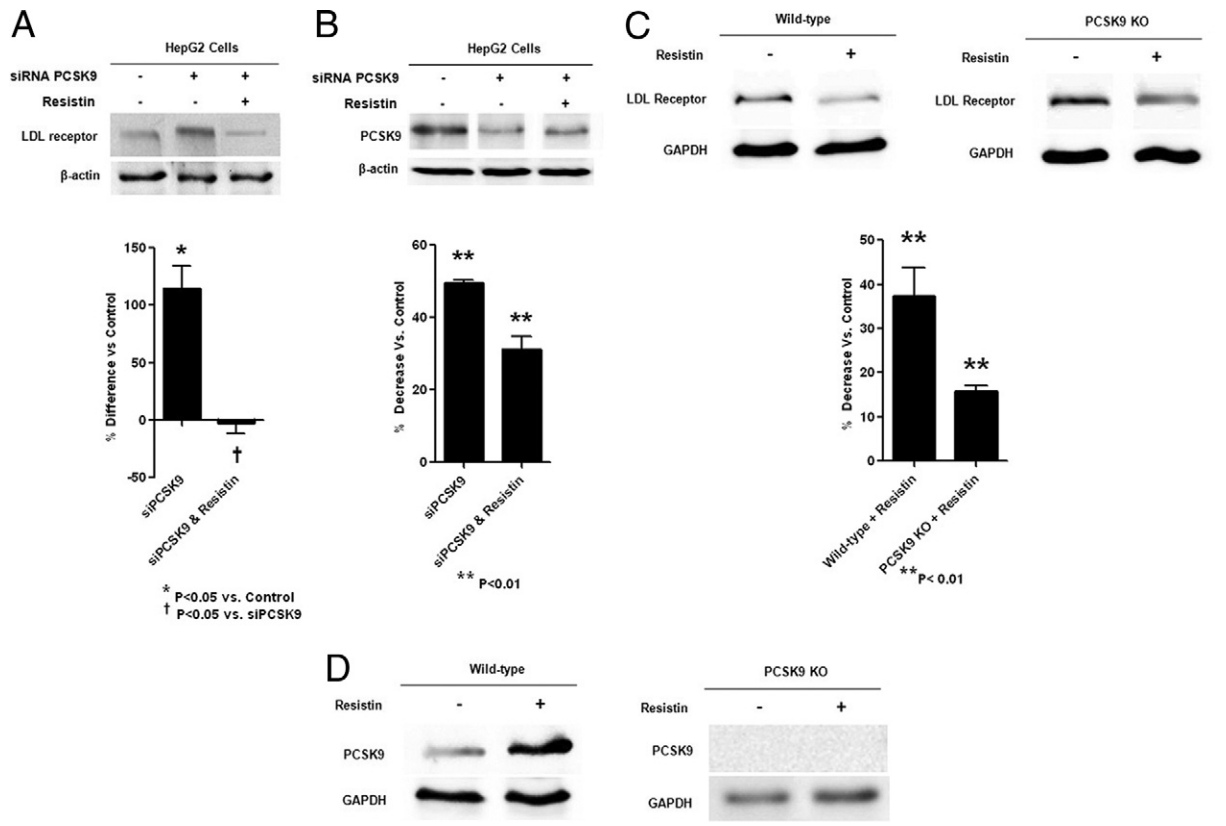

Figure 3 **Determination of the Contribution of PCSK9 in the Resistin-Mediated (50 ng/ml, 24 h) Reduction in Hepatocyte LDLR Protein Expression**

(A) Resistin reversed the up-regulation of LDLR protein expression in HepG2 cells induced by PCSK9 small interfering ribonucleic acid (siRNA) treatment. **(B)** PCSK9 siRNA treatment caused a reduction in human hepatocyte PCSK9 protein levels when added to hepatocytes alone and a lesser reduction when resistin was added together with PCSK9 siRNA. **(C)** When hepatocytes isolated from wild-type mice were treated with resistin, a reduction in LDLR levels was observed. When hepatocytes isolated from PCSK9 knockout mice were treated similarly with resistin, a lesser reduction in LDLR levels occurred. **(D)** An increase in cellular PCSK9 protein levels were observed when wild-type hepatocytes were treated with resistin. GAPDH = glyceraldehyde-3-phosphate dehydrogenase; other abbreviations as in Figure 2.

obese human serum reduced PCSK9 expression by 48 ± 6%, and in lean human serum, it reduced it by 38 ± 5% ($p < 0.01$ for both) (Fig. 5E).

Effect of resistin on statin-mediated up-regulation of hepatocyte LDLR levels. We tested whether resistin inhibits the normal statin-mediated up-regulation of hepatocyte LDLR levels. Resistin (50 ng/ml, 24 h) diminished the increase in hepatocyte LDLR expression induced by lovastatin treatment (5 μM, 24 h) by 60% ($p < 0.01$) (Fig. 6A) and increased cellular PCSK9 protein by 55% versus lovastatin treatment alone ($p < 0.01$) (Fig. 6B).

Discussion

Multiple studies have consistently shown a strong independent relationship between serum resistin levels and LDL concentrations in obese humans, demonstrating the relevance of studying resistin–LDL interactions in a human context (27,28). We further found for the first time that human resistin treatment of human hepatocytes directly causes dose-response reductions in hepatocyte LDLR protein levels. The maximum LDLR reduction (of 40%) was reached at concentrations of resistin characteristic of obese individuals (50 and 75 ng/ml) (19).

We then sought to determine the mechanisms by which resistin inhibits hepatocyte LDLR protein expression. Essentially, a dual control system of hepatic LDLR expression is currently recognized (13,14). On the synthesis end, the transcription factor, SREBP2, stimulates LDLR mRNA expression, usually when hepatocytes are depleted of cholesterol (13,14). The recently identified secreted hepatocyte protein of high current interest, PCSK9, then mediates post-transcriptional LDLR protein degradation (13,14,29,30). PCSK9 binds to the extracellular domain of the LDLR on the cell surface and then targets the LDLR, after endocytosis, for intracellular lysosome-mediated degradation instead of recycling to the plasma membrane (13,14).

We found that resistin treatment of HepG2 cells increases cellular mRNA expression of SREBP2 and

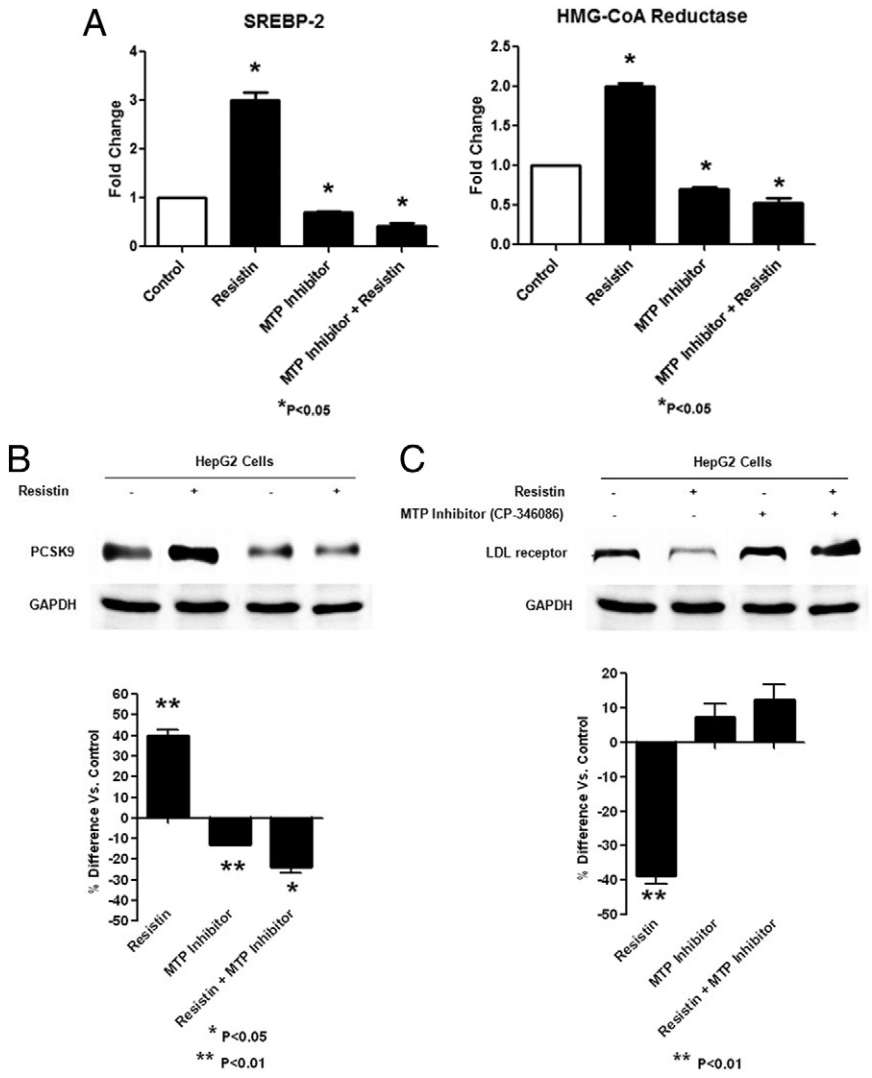

Figure 4 **Hepatocyte LDLR and PCSK9 Protein Expression by Resistin Treatment (50 ng/ml, 24 h) of HepG2 Cells in the Presence of an MTP inhibitor, CP-346086**

(A) Treatment of HepG2 cells with a microsomal triglyceride transfer protein (MTP) inhibitor, CP-346086 (1.3 nmol/l), induced a significant reduction in cellular de novo cholesterol synthesis, mediated by a reduction in SREBP2 mRNA expression and its target gene, 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase, rate-limiting in cellular cholesterol biosynthesis. **(B)** The addition of CP-346086 to hepatocytes treated with resistin reversed the increase in PCSK9 protein levels induced by resistin and, moreover, caused a significant decrease in PCSK9 protein compared with untreated control cells. **(C)** Treatment of human hepatocytes with CP-346086 reversed the decline in LDLR protein levels observed with resistin treatment. Other abbreviations as in Figures 2 and 3.

SREBP2 target genes, including HMG-CoA reductase, the rate-limiting protein in intracellular cholesterol biosynthesis. The result of this increase in de novo cellular lipid biosynthesis mediated by resistin was an increase in intracellular neutral lipid content, particularly a 25% increase in hepatocyte triglycerides and a 20% increase in cholesteryl esters. Resistin also stimulated the mRNA expression of PCSK9, also an SREBP2 target gene (29), which was reflected in resistin-mediated significant increases in hepatocyte PCSK9 protein levels.

We further investigated the extent of the role of PCSK9 in the resistin-mediated reduction in hepatocyte LDLR levels. We therefore inhibited PCSK9 gene expression in hepatocytes via PCSK9 siRNA treatment for 24 h. The addition of resistin reversed the marked more than 100% elevation in hepatocyte LDLR expression induced with PCSK9 siRNA treatment and also enhanced cellular PCSK9 protein levels, compared with siRNA treatment alone. This finding suggests that resistin stabilizes hepatocyte PCSK9 protein. Overall, these findings indicate that the reduction in cellular LDLR protein levels by resistin and enhanced LDLR degradation occur, at least in part, via up-regulation of PCSK9. This is a novel function of human resistin and is the first identification of a natural

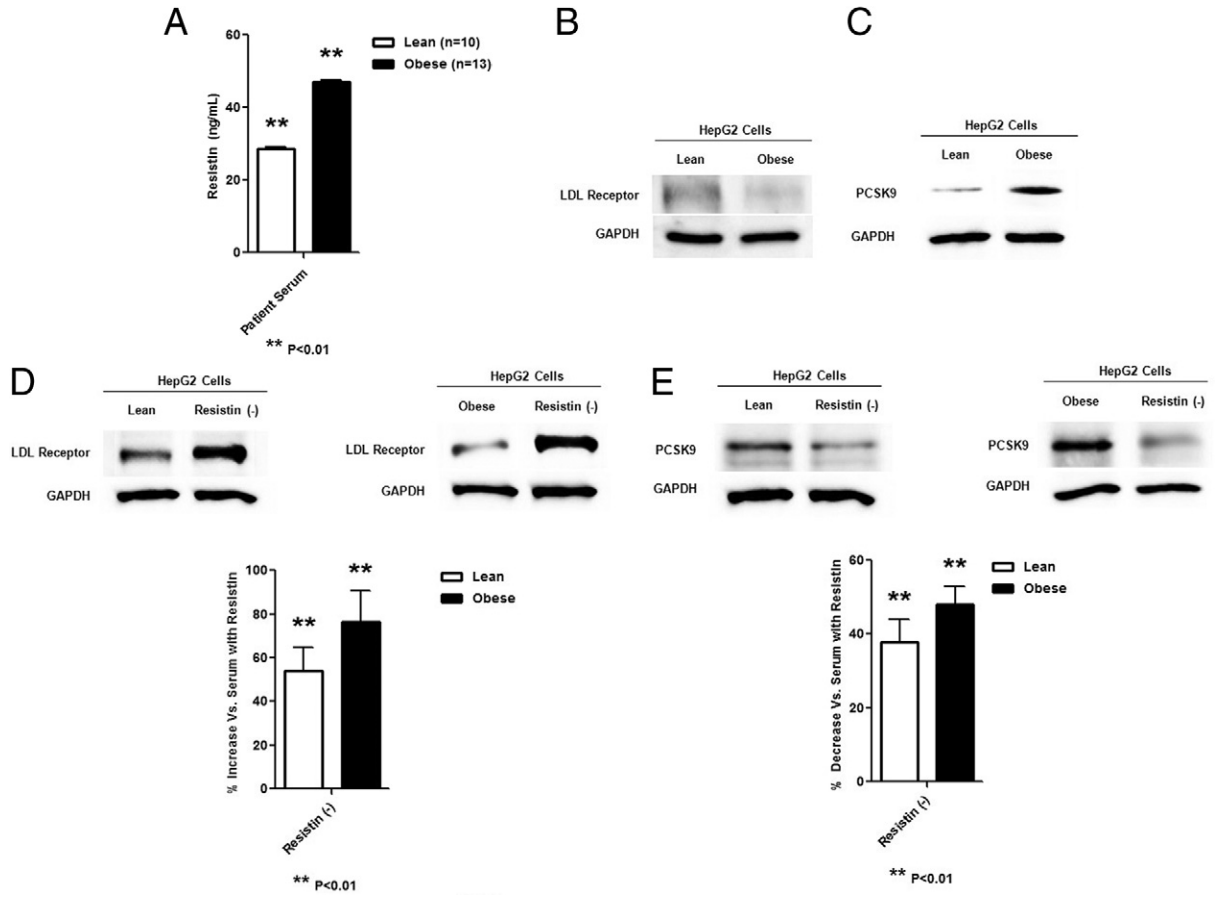

Figure 5 The Role of Resistin on Human Hepatocyte LDLR and PCSK9 in a Physiologically Relevant Human Setting

(A) Serum resistin levels were elevated in obese human serum (n = 13) compared with lean human serum (n = 10). **(B)** Obese human serum (10% in Dulbecco's modified Eagle's medium [DMEM], 24 h), with elevated resistin levels, had a significant inhibitory effect on HepG2 LDLR expression compared with hepatocytes incubated with lean human serum (10% in DMEM, 24 h). **(C)** Obese human serum caused a greater induction in hepatocyte PCSK9 expression compared with lean human serum. **(D)** Antibody-immunoprecipitation removal of resistin from obese serum caused a greater increase in HepG2 LDLR expression compared with removal of resistin from lean serum. **(E)** The amelioration of hepatocyte LDLR levels with antibody-immunoprecipitation removal of resistin from both obese and lean serum was attributed, at least in part, to reduction in hepatocyte PCSK9 protein expression. Abbreviations as in Figures 2 and 3.

serum factor directly regulating PCSK9 protein levels in hepatocytes.

To more directly quantify the role of PCSK9 in the resistin-mediated reduction in hepatocyte LDLR levels, hepatocytes isolated and cultured from wild-type mice were treated with resistin, and we then compared them with resistin-treated hepatocytes from PCSK9 knockout mice. In wild-type mice hepatocytes, resistin markedly decreased LDLR protein levels by 40%, compared with untreated hepatocytes, and also increased PCSK9 levels significantly, similar to our findings in human hepatocytes. Resistin also significantly decreased LDLR expression in hepatocytes from PCSK9 knockout mice, but the magnitude of the effect was reduced to a 15% decline in LDLR expression compared with untreated hepatocytes from PCSK9 knockout mice. These findings show that the elevation in PCSK9 protein levels induced by resistin plays a major, but not exclusive, role in the reduction of cellular LDLR levels mediated by resistin.

We next used inhibitors of MTP (26) to determine if excess accumulation of intracellular lipids through MTP inhibition would ameliorate the cellular up-regulation of SREBP2 and its targets, particularly PCSK9, induced by resistin, thereby ameliorating the effects of resistin. MTP inhibition, via CP-346086, induced a significant reduction in cellular SREBP2 mRNA expression, and it thereby reversed the increase in PCSK9 protein levels induced by resistin and thus reversed the decline in LDLR protein levels observed with resistin. These results indicate that the SREBP2-mediated elevation in hepatocyte PCSK9 levels contributes to the decline in cellular LDLR levels induced by resistin.

We wished to further determine if, in a physiologically relevant human setting, resistin inhibits hepatocyte LDLR protein expression. We therefore incubated human hepatocytes

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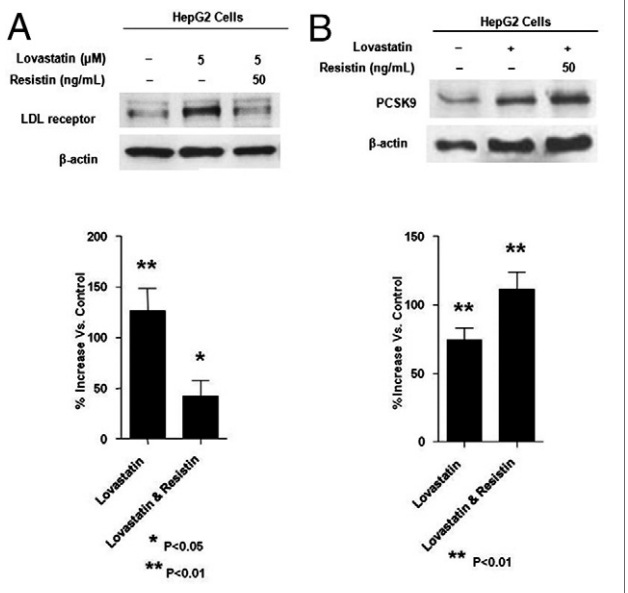

Figure 6 **The Effect of Resistin (50 ng/ml, 24 h) on Statin-Mediated LDLR Regulation in HepG2 Cells**

(A) Lovastatin treatment of HepG2 cells (5 μM, 24 h) significantly increased cellular LDLR levels, as expected. The addition of resistin to lovastatin greatly diminished the statin-mediated up-regulation of cellular LDLR levels. **(B)** The addition of resistin to lovastatin increased cellular PCSK9 levels. Abbreviations as in Figures 2 and 3.

with serum from healthy obese males (BMI >30 kg/m^2 and <35 kg/m^2 and waist circumference >102 cm) with high resistin concentrations (60% elevated compared with lean subjects) and compared them with hepatocytes incubated with serum from lean males (BMI ≤25 kg/m^2 and waist circumference <102 cm). The results demonstrated a greater inhibitory effect of obese human serum on cellular LDLR protein expression than lean serum incubation of hepatocytes, concomitant with a greater elevation in PCSK9 levels.

We then performed antibody-immunoprecipitation removal of resistin from human serum and examined the subsequent effect on hepatocyte LDLR expression. Antibody removal of resistin in obese human serum reversed the obese serum–mediated reduction in cellular LDLRs remarkably (by 80%), an effect that was mediated in part by reduced PCSK9 expression (by 50%). Removal of resistin in lean human serum also increased hepatocyte LDLR levels, albeit to a lesser extent. These results indicate that resistin in human serum plays a quantitatively important role in mediating hepatocyte LDLR levels. This further indicates that reduction or inhibition of serum resistin in humans is a potentially effective treatment for elevated LDL, particularly in obese states.

The major class of drugs currently administered to patients with elevated serum LDL are statins. Statins function by reducing cellular cholesterol levels; this reduction activates SREBP2, leading to the transcriptional activation of the LDLR (13). Because resistin stimulates PCSK9, the resistin-mediated increase in PCSK9 expression, which should be more prevalent in obese individuals, may attenuate the increase in LDLR expression in patients administered statins. Consistent with this concept, the evidence suggests that obese individuals are less likely to achieve optimal LDL lowering with statin therapy (31–34).

We therefore tested whether resistin inhibits the normal statin-mediated up-regulation of hepatocyte LDLR levels. Resistin diminished the increase in hepatocyte LDLR expression induced by lovastatin treatment (5 μM) considerably (by 60%). This inhibition of the statin-induced LDLR up-regulation by resistin was attributed in part to increased cellular PCSK9 protein levels (by 55%). These results indicate that inhibitors of resistin may act synergistically with statins to enhance hepatocyte LDLR expression and reduce plasma LDL levels.

In general, there is considerable controversy on the role of resistin in the metabolic complications of obesity. Although correlations between serum resistin and LDL levels in humans have been shown in multiple reports (27,28), no causal role of resistin in LDL regulation has previously been shown in humans. Here we have demonstrated that human resistin robustly inhibits human hepatocyte LDLR protein expression. We have further identified that PCSK9-mediated enhanced LDLR degradation is a major, but not exclusive, mechanism by which resistin exerts its inhibitory effect on cellular LDLR levels. We have also shown a direct effect of resistin on LDLRs, independent of PCSK9, in our experiments with resistin treatment of hepatocytes isolated from PCSK9 knockout mice. Analysis of the crystal structure of PCSK9 has showed, intriguingly, that PCSK9 and resistin share a fully folded C-terminal cysteine-rich domain (35). It has thus been suggested that resistin and PCSK9 compete for or share a common receptor, likely the LDLR, which will need to be discerned in future studies in in vitro and in vivo settings. In addition to the aforementioned mechanistic insights, our studies with resistin suggest that in a clinical setting, elevated resistin in obese humans may mask the full potential of statins in LDL lowering, which has important implications on patient segregation and targeted drug therapy. This will need to be specifically tested in future clinical studies.

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Key Words: dyslipidemia ■ hepatic ■ LDL receptor ■ obesity ■ resistin.

APPENDIX

For an expanded Methods section, please see the online version of this article.